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JMJD5 functions in concert with TOC1 in the Arabidopsis circadian system

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The circadian clock modulates the expression of approximately one-third of Arabidopsis genes and as such plays a central role in the regulation of plant metabolism and responses to the environment. We have recently identified a novel component of the Arabidopsis circadian clock, JMJD5, based on its coexpression with TOC1, an evening-phased component of the molecular oscillator. We now examine the genetic interaction between *TOC1* and *JMJD5* in greater detail and demonstrate that *toc1* is not epistatic to *jmjd5*, suggesting that these two proteins act in closely linked but parallel genetic pathways. The human homolog of JMJD5, KDM8, has been shown to have histone demethylation activity and is able to partially rescue the plant *jmjd5* circadian phenotype. The potential role of JMJD5 as a histone demethylase within the circadian clock is discussed.

Key words: circadian clock, histone demethylase, KDM8, JMJ30, TOC1

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The Arabidopsis Circadian Clock

Most organisms experience daily modulations of their environment induced by the rotation of the Earth on its axis. These predictable changes have led to the repeated evolution of endogenous molecular timers, known as circadian clocks, in diverse lineages.[1] Such internal biological rhythms permit anticipation of regular cues of dawn and dusk and are used to regulate gene expression, protein stability and other, higher order cellular functions.[2] While the circadian clock is entrained to environmental cues such as changes in light and temperature, endogenous rhythms persist for multiple cycles without external input.[3]

The Arabidopsis circadian clock is comprised of multiple interlocking feedback loops,[2] with one transcriptional circuit consisting of two partially redundant MYB transcription factors, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) that promote the expression of a third component, *TIMING OF CAB1 EXPRESSION* (*TOC1*).[4] *TOC1* represses *LHY* and *CCA1* expression via a partially described mechanism involving additional factors including *CCA1 HIKING EXPEDITION* (*CHE*),[5] thereby forming a negative feedback loop. To further characterize genes acting in concert with *TOC1* we used a data mining strategy to examine previously published miroarray data for genes coexpressed with *TOC1*. This analysis and subsequent characterization revealed *JMJD5*, a putative histone demethylase, to be a novel factor within the Arabidopsis circadian clock.[6]

TOC1 and JMJD5 Act in Partnership to Regulate the Circadian Clock

We have shown that *jmjd5-1 toc1-2* mutants have a synergistic mutant phenotype compared to either single mutant.[6] However, interpretation of these data is complicated by the partial loss-of-function nature of the *toc1-2* allele, which produces low levels of correctly-spliced *TOC1* transcript.[7] The synergistic interaction between *jmj5-1* and *toc1-2* could therefore indicate either that JMJD5 and TOC1 act in parallel pathways, or that JMJD5 affects clock function via the small amount of functional

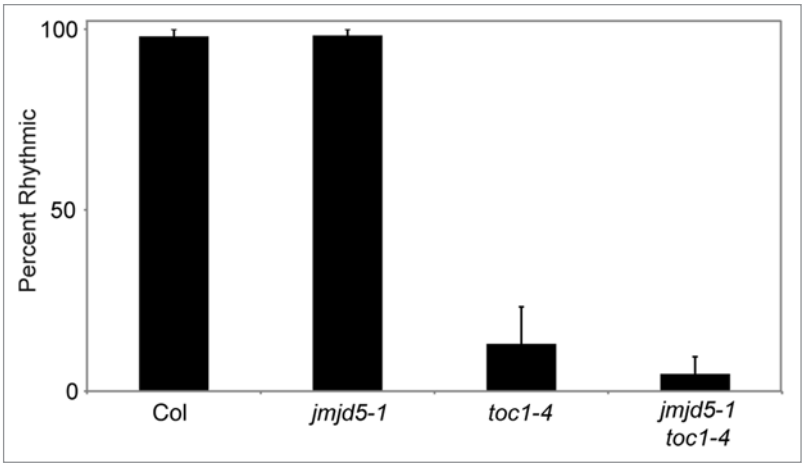

Figure 1. Circadian rhythmicity of *toc1-4* and *jmjd5-1 toc1-4* seedlings in constant red light. Percent rhythmicity was defined as the fraction of seedlings that returned a period estimate with a relative amplitude error (RAE) <1 as determined by FFT-NLLS.[20] Plants were entrained to 12:12 LD cycles for six days before being moved to continuous 30 μmol m^{-2} s^{-1} red light. Error bars indicate SE from three independent experiments.

TOC1 protein produced in the *toc1-2* background.

To help resolve this point we crossed the *jmjd5-1* allele to *toc1-4*, a true *TOC1* null allele,[8] and assessed the circadian phenotypes of the resultant homozygous mutant progeny. Consistent with *toc1-4* being a more severe allele than *toc1-2*, only 13% of *toc1-4* plants assayed had a detectable circadian rhythm under constant red light (**Fig. 1**), in contrast to *toc1-2* in which 70% of plants displayed rhythmic luciferase activity.[6] Only 5% of *jmjd5-1 toc1-4* double mutants were rhythmic, compared to 30% of *jmjd5-1 toc1-2* plants (**Fig. 1** and reviewed in ref. 6). Although there was a modest decrease in rhythmicity in *jmjd5-1 toc1-4* seedlings compared to *toc1-4* alone (**Fig. 1**), this decrease was not significant, most likely due to the generally poor cycling observed in the *toc1-4* single mutant. These data reinforce the conclusion that *toc1-2* plants retain a modicum of TOC1 activity that appears responsible for the retention of circadian rhythms in these plants under constant red light.

We have previously demonstrated that *toc1-2* mutant phenotypes are less severe under constant red and blue light than under constant red light alone (**Fig. 2A** and reviewed in ref. 6) and we therefore used this light condition to further characterize our *jmjd5-1 toc1-4* mutant plants. *toc1-4* mutants demonstrated a greatly shortened circadian rhythm compared to wild type under these conditions (18.21 h vs. 24.34 h, respectively, **Fig. 2B and C**), but these rhythms were relatively robust (**Fig. 2B and C**). In comparison, *jmjd5-1 toc1-4* seedlings were either arrhythmic or had very poor rhythms (**Fig. 2B and C**). This exacerbation of the *toc1-4* phenotype upon loss of JMJD5 function suggests that JMJD5 does not merely modify TOC1 activity but that both proteins act in parallel to modulate gene expression. Future work will determine whether JMJD5 and TOC1 activities converge on the regulation of morning-phased circadian genes as suggested by our previous work in reference 6.

The Role of Histone Methylation within the Circadian Clock

Changes in chromatin structure may be precipitated by either histone acetylation or methylation, epigenetic modifications which were originally described as permanent but are now recognized as being much more dynamic marks of gene expression.[9,10] Several studies have demonstrated that chromatin states of circadian clock genes may change over circadian time (reviewed in ref. 11 and 12). Interestingly, we have found that not only does disruption of *JMJD5* affect clock function in Arabidopsis, but that human cells deficient for the ortholog of this gene also have a circadian phenotype.[6] We found that the plant and human ortholog are at least partially functional in each reciprocal system.[6] The human ortholog, *HsJMJD5/KDM8*, can demethylate dimethylated lysine residues in position 36 of histone H3 (H3K36me2),[13] which raises the possibility that JMJD5 modulates gene expression in both Arabidopsis and human systems through this enzymatic activity. In support of this notion, JMJD5 (also known as JMJ30) fused to GFP accumulates in both the nucleus and cytoplasm.[14] It will be interesting to determine whether cytoplasmic JMJD5 has a functional role and whether JMJD5 localization is dependent on other factors as reported for TOC1.[15]

H3K36me2 marks at the 3' regions of Arabidopsis genes have recently been correlated with a more 'closed' chromatin structure and are thought to help ensure the fidelity of transcription by preventing the inappropriate use of cryptic promoters.[16] Similarly, increased H3K36 methylation at transcriptional start sites has been correlated with a reduction in transcript initiation in yeast.[17,18] JMJD5 may therefore help maintain histone marks for the appropriate transcription of central clock associated genes. Such an interpretation would fit with data from ourselves and others showing perturbation of central circadian gene expression only under high fluence rates of monochromatic red light.[6,14] The mechanism by which this 'high red' phenotype is induced is a topic for speculation; light has previously been shown to induce changes in histone modifications[19] and it is therefore possible the increased fluence rates used in our assays altered histone modification so as to magnify the *jmjd5* mutant phenotypes. Further work will be required to elucidate the mechanism by which JMJD5 contributes to the correct cycling of the circadian clock in plants and humans.

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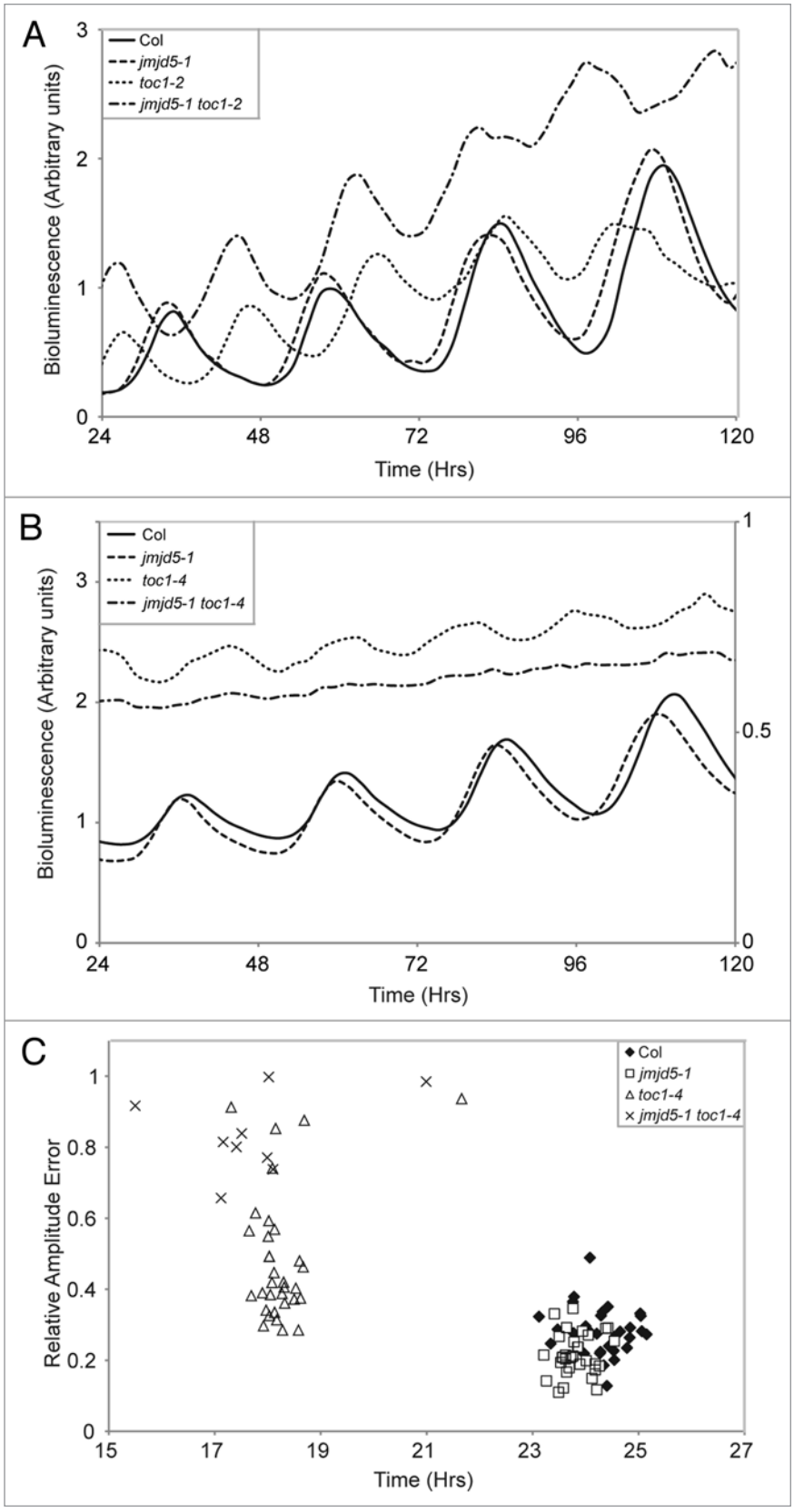

Figure 2. Comparison of jmjd5-1 and toc1 circadian rhythms in constant red and blue light. (A) Average bioluminescence of seedlings expressing luciferase under the control of the CCR2 promoter (CCR2::LUC) in wild-type columbia (col, solid), jmjd5-1 (dashed), toc1-2 (dotted) and jmjd5-1 toc1-2 (dash-dot) genetic backgrounds. Plants were entrained to 12:12 light:dark cycles for 6 days before being monitored in continuous red and blue light (15 μmol m^{-2} s^{-1} red and 20 μmolm^{-2} s^{-1} blue). Error bars represent SE, n ≥ 20. (B) Average luciferase activity of seedlings expressing luciferase under the control of the CCR2 promoter (CCR2::LUC) reporter lines in wild type (col, solid), jmjd5-1 (dashed), toc1-4 (dotted) and jmjd5-1 toc1-4 (dash-dot) backgrounds. toc1-4 and jmjd5-1 toc1-4 traces are plotted on a secondary axis for clarity. Plants were treated as described in (A). Presented data are representative of three independent experiments. (c) Scatter plot showing period and RAE estimates of wild-type columbia (col, diamond), jmjd5-1 (square), toc1-4 (triangle) or jmjd5-1 toc1-4 (cross) genotypes. Seedlings that did not return a period estimate were excluded, data points are replotted from (B).

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